Meta-analysis of antiviral protection of white spot syndrome virus vaccine to the shrimp

Shu-Ying Feng[a†], Gao-Feng Liang[a†], Zheng-Shun Xu[a], Ai-Fang Li[a], Jing-Xia Du[a], Guan-Nan Song[a], Shai-Yu Ren[a], Yu-Lin Yang[a], Guanglong Jiang[b,c]*

[a]*Medical College, Henan University of Science and Technology, Luoyang, Henan 471023, China*

[b] *Center for Computational Biology and Bioinformatics, Indiana University School of Medicine, Indiana 46202 USA*

[c] *Department of BioHealth Informatics, Indiana University-Purdue University Indianapolis, Indiana 46202 USA*

†These authors contributed equally to this work.

*C**orresponding author**

Center for Computational Biology and Bioinformatics,

Indiana University School of Medicine,

Indiana 46202, USA

Department of BioHealth Informatics,

Indiana University Purdue University Indianapolis,

Indiana 46202 USA

E-mail: ggjiang@iu.edu

Abbreviations

WSSV, white spot syndrome virus; RNAi, RNA interference; *E. coli, Esherichia coli*; *B. subtili, Bacillus subtili*; *D. salina, Dunaliella salina*;

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Abstract: Currently, white spot syndrome virus (WSSV) is one of the most serious pathogens that impacts shrimp farming around the world. A WSSV vaccine provides a significant protective benefit to the host shrimp. Although various types of vaccines against WSSV have emerged, the immune effects among them were not compared, and it remains unclear which type of vaccine has the strongest protective effect. Meanwhile, due to the lack of effective routes of administration and immunization programs, WSSV vaccines have been greatly limited in the actual shrimp farming. To answer these questions, this study conducted a comprehensive meta-analysis over dozens of studies and compared all types WSSV vaccines, which include sub-unit protein vaccines, whole virus inactivated vaccines, DNA vaccines and RNA-based vaccines. The results showed that the RNA-based vaccine had the highest protection rate over the other three types of vaccines. Among the various sub-unit protein vaccines, VP26 vaccine had the best protective effects than other sub-unit protein vaccines. Moreover, this study demonstrated that vaccines expressed in eukaryotic hosts had higher protection rates than that of prokaryotic systems. Among the three immunization modes (oral administration, immersion and injection) used in monovalent protein vaccines, oral administration had the highest protection rate. In natural conditions, shrimp are mostly infected by the virus orally. These results provide a guide for exploration of a novel WSSV vaccine and help facilitate the application of WSSV vaccines in shrimp farming.

Key words: WSSV; Vaccine type; Immune program; Virus gene; Protection rate; Meta-analysis

1. Introduction

Since the outbreak in shrimp in the 1990s, white spot syndrome virus (WSSV) has become the most virulent pathogen in the industry and impacts shrimp farming globally each year [1]. As a result, the development of viral vaccine has become one of intense focus of research in this field. So far, research in WSSV vaccine has made great progress and a variety types of vaccines have emerged, such as sub-unit protein vaccine [2], inactivated whole virus vaccine [3], DNA vaccine [4], RNA-based vaccine [5] and so on. These vaccines have proven that they can significantly enhance the immune response of the host shrimp and provide a significant protective effect in shrimp [6–9]. However, the protection rate among vaccines has not been compared. For the same viral protein, taking VP28 as an example, different types of vaccines have different immune effects in the host [10–12]. Additionally, even with same vaccine, different modes of immunization have different immune protective effects in animals [7, 8, 13]. Furthermore, different immunization times or attack times also result in different immune effects for the same protein vaccine [14, 15]. Conflicting conclusions were also observed between different studies [16, 17]. Furthermore, the immunization program, which includes the immune time and length, time of virus attack, mode of virus attacks and so on has a decisive effect on the immune effects. Although a WSSV vaccine has the potential significantly benefit the host shrimp, its practical application is heavily hindered by the lack of efficient and uniform immunization programs. To solve these above-mentioned issues, this study conducted a comprehensive meta-analysis for all kinds of current WSSV vaccines.

2. Materials and Methods

2.1 Search strategy and data retrieval

All the relevant studies were retrieved using the search keywords of ‘white spot syndrome virus’ plus ‘vaccine’, or ‘immune’, or ‘protection’, or ‘antiviral’, or ‘control’, or ‘prevention’ etc. Search domains came from the English scientific publication databases such as the PubMed, SCI database, Elsevier, and the Springer; Chinese scientific databases like the CQVIP database, Wanfang database, China national knowledge infrastructure (CNKI), Chinese science citation database (CSCD), and also

from the other public search engines, such as Google scholar and Web of Science. There were 178 articles related to these keywords in which 98 references were determined to be relevant after review. After analysis, 54 publications containing protective effects fit the criteria for inclusion. All the data used in this study were extracted from the results of these original research papers. The WSSV vaccines were divided into four main categories; viral sub-unit vaccines, inactivated whole virus vaccines, DNA vaccines, and RNA-based vaccines. The characteristics of each vaccine were summarized respectively in supplementary data 1–4, with regard to the different types of vaccine, forms of the protein subunits, modes of administration, protection rate, and other relevant data [3–10, 12–14, 16–55].

2.2 Statistical analysis

The outcome used in this study was the proportion of protection rate against WSSV infection among the different types and modes of vaccines. A Freeman-Tukey transformation [56] was applied to the protection rate to stabilize the variance and make the 0 to 1 ranged proportion more suitable for the statistical comparisons among groups. Heterogeneity was assessed using the Cochran's Q statistics, and subset and regression analysis were conducted to explore potential sources of heterogeneity. A multivariate logistic regression was conducted to model the effects of different types of vaccines and virus attack modes. The analyses were conducted in statistical environment R (version 3.4.0) using *metafor* package [57].

3. Results

3.1 Meta-analysis of overall protection rates for main types of vaccines

To estimate the average protective effects for each type of vaccine, we first applied the meta-analysis random-effects model to protection rates of studies in the four main vaccine subtypes separately. Figure 1 shows the forest plot for protective rates of each study, and the weighted estimate of average protection rates in the context of a random-effects model for each subtype of vaccines. The weighted protection rates for each one of those four subtypes of vaccines, ranking from high to low, are RNA-based vaccine (Fig 1D, 80.18%), inactivated whole virus vaccine (Fig 1B, 65.29%), DNA

vaccine (Fig 1C, 59.00%) and sub-unit protein vaccine (Fig 1A bottom, 55.88%) respectively. The monovalent (Fig 1A top) and polyvalent (Fig 1A middle) sub-unit protein vaccines have average protection rates 56.12% and 55.20%, respectively. Among those types of vaccines, the RNA-based vaccine has the highest protection rates and the smallest heterogeneity. Conversely, monovalent protein vaccines (Fig 1A top) and DNA vaccines (Fig 1C) have a very large heterogeneity ($p<0.001$), and polyvalent vaccines (Fig 1A middle) have the lowest average protection rate (55.20%). Figure 1E wholly shows the side by side comparison of the estimated protection rates for monovalent, polyvalent, while virus, DNA vaccine and RNA-based vaccines.

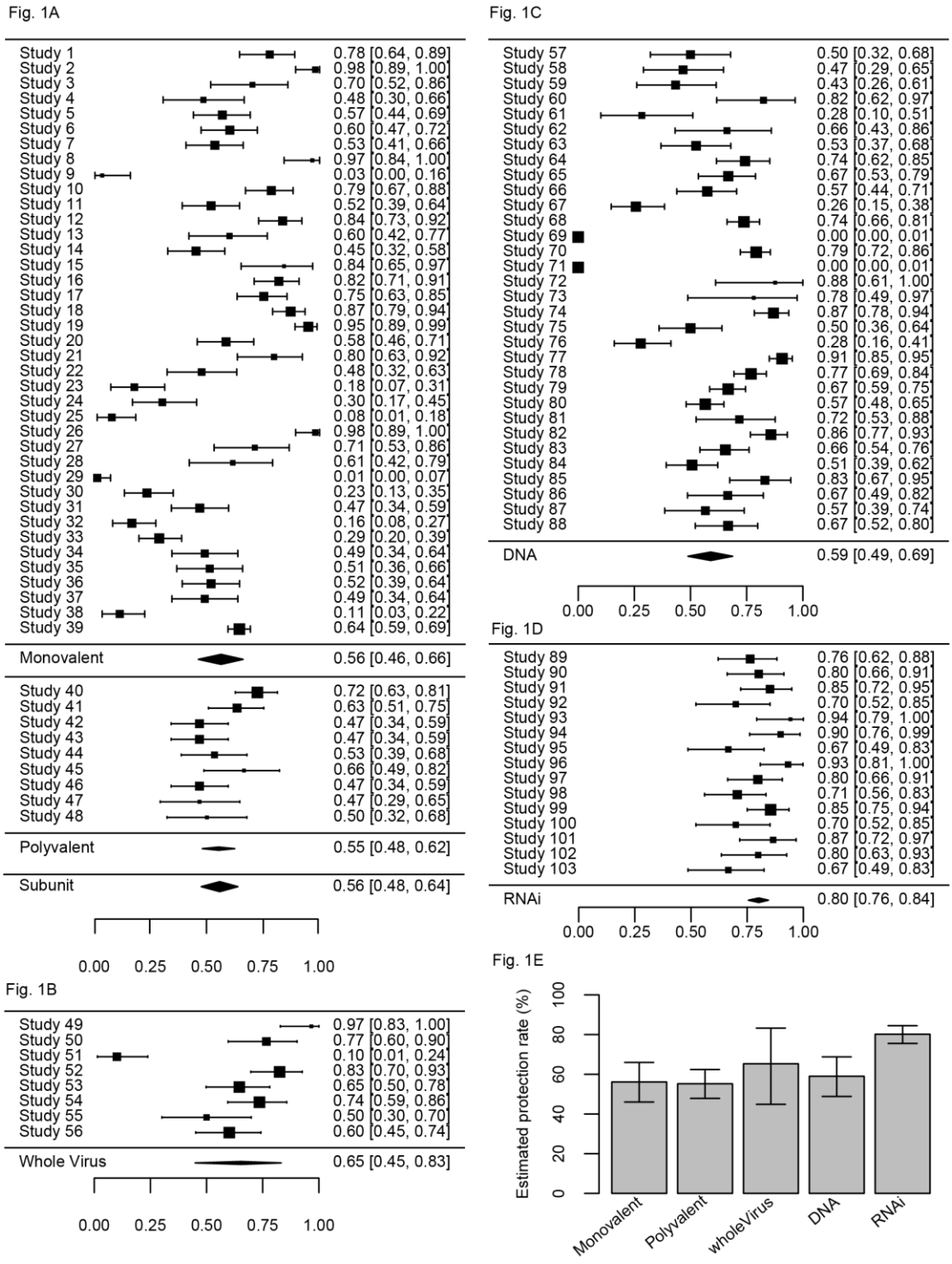

Fig. 1. Forest plot of protective rates for four main vaccine types and the weighted estimated average protection rates. (1A) Subunit vaccines (top: monovalent vaccines; middle: polyvalent vaccines; bottom: estimated average protection rate for subunit vaccines); (1B) Whole virus inactivated vaccines; (1C) DNA vaccines; (1D) RNA technology-based vaccines; (1E) Estimated protection rates with confidence intervals for each type of vaccine.

3.2 Monovalent Vaccines

3.2.1 Subgroup analysis

Among the subtype of vaccines, monovalent vaccines have the largest number of published studies (n=39) and the greatest heterogeneity. 64% of monovalent vaccine studies were conducted with the protein VP28 (Fig 2A), with an average protection rate of 63.21%, while still a high level of heterogeneity ($p<0.001$). There are only 3 studies conducted with VP26 protein (Fig 2B), which had the highest estimated protection rate (80.48%). 7 studies using VP19 proteins revealed the lowest average protection rate of 28.49% (Fig 2C). Beyond those three proteins, little publish research has been conducted (Fig 2D) and that data was not meta-analyzed here. Among the 25 studies using VP28, 14 of them expressed this protein in *E. coli* had relatively low estimated protection rate (52.32%) and high heterogeneity; on the contrary, 11 studies using other expression hosts such as *B. subtilis* and Baculovirus had a higher estimated protection rate (75.73%, Fig. 3).The meta-analysis above showed that the type of proteins used in vaccines and expression host contributed to part of the heterogeneity (Fig 2 and Fig 3).

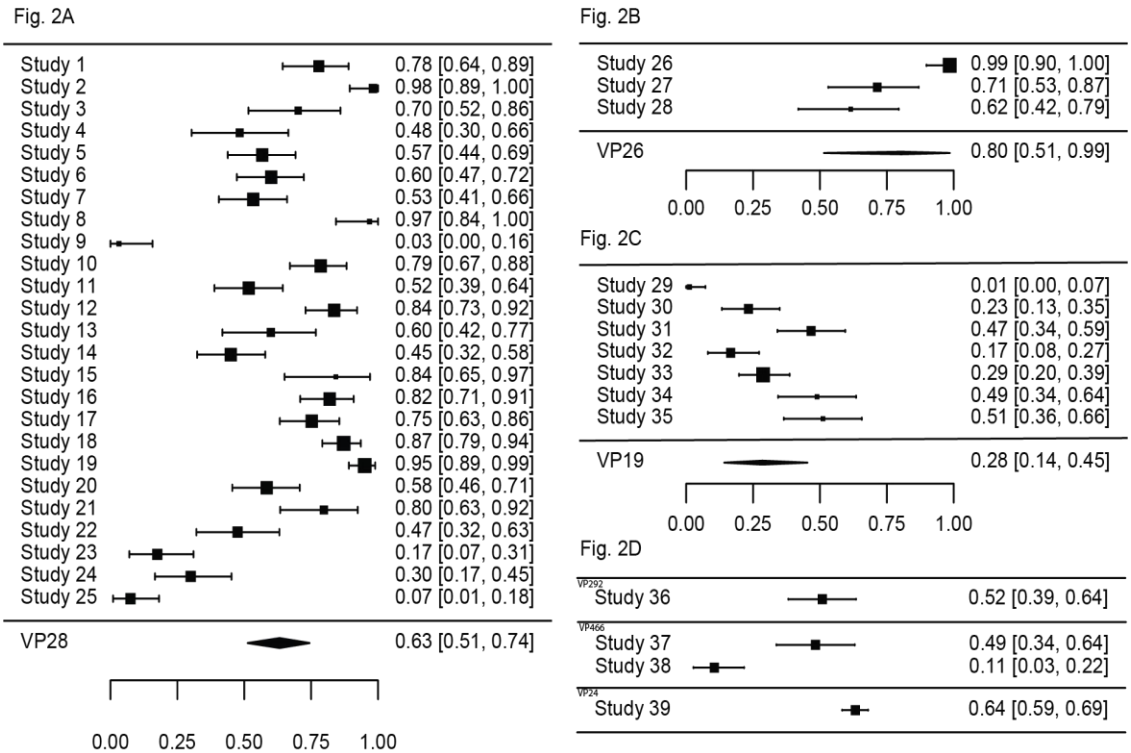

Fig. 2. Forest plot of average protection rates for different monovalent vaccines. (2A) Monovalent VP28 vaccine; (2B) Monovalent VP26 vaccine; (2C) Monovalent VP19 vaccine; (2D) Monovalent other WSSV envelope vaccines.

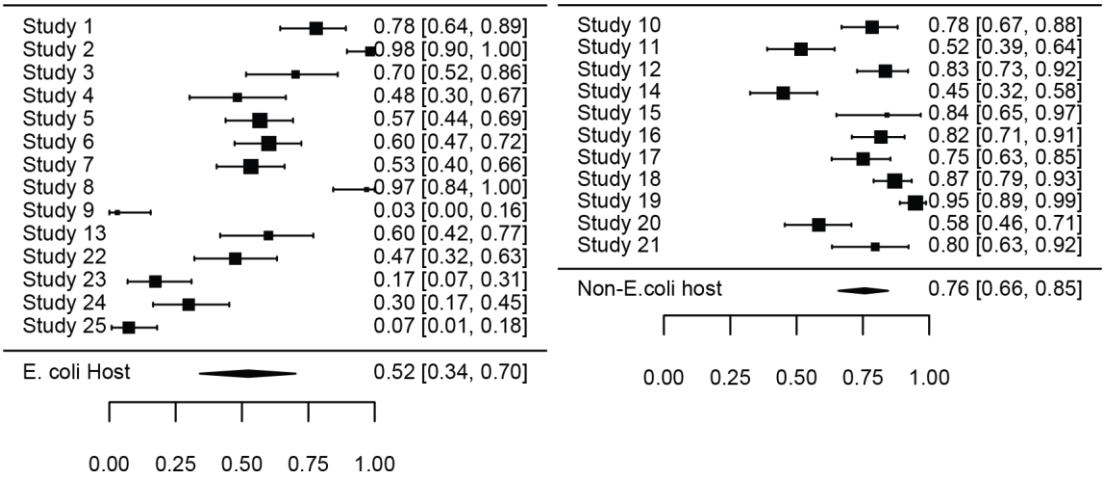

Fig. 3. Forest plot of average protection rates for monovalent vaccine studies. (Left) shown that conducted on the host of *E. coli;* (Right) shown that conducted on the host of non-*E. coli*.

3.2.2 Regression analysis

The logistic regression was applied to the monovalent vaccine data including 39 studies and 2,205 experimental units. Univariate regression analysis confirmed that all five factors are significantly associated with protection rates (Table 1), while in the multivariate analysis, protein form, immunization mode, and virus attack mode were no longer significantly associated with protection rates because of the collinearity among them (Table 2). From the univariate analysis, VP26 had a higher protection rate than protein VP28, while VP19 and VP466 had relatively low protection rates compared to VP28. VP292 also had an inferior protection rate compared to VP28 , while VP24 was not different from VP28.

Table 1 Univariate analysis of factors that associate with protection rate

Variables	P value
Protein (VP28, VP19, VP24, VP26, VP292 and VP466)	$P<2.2\times10^{-16}$
Proteins Form (Purified, Transgenic and Others)	2.8×10^{-15}
Expression host (*E. coli* and Others)	4.0×10^{-8}
Immunization mode (Oral, Immersion and Injection)	2.4×10^{-8}
Virus attack mode (Oral, Immersion and Injection)	7.5×10^{-6}

Table 2 Multivariate analysis of factors associate with protection rate

Variables remain significant	P value
Protein (VP28, VP19, VP24, VP26, VP292 and VP466)	$P<2.2\times10^{-16}$
Expression host (*E. coli* and Others)	$P<2.2\times10^{-16}$

The mentioned-above trends can also be found in the boxplot of protection rates against every protein (Fig 4A). Among three protein forms, purified protein has a significantly lower protection rate than transgenic or other forms (Fig 4B). Fig 4C suggests that protein vaccines expressed in *E. coli* had lower protection rate than other expression hosts. As to the effect of immunization modes, Fig 4D shows that oral differs significant from immersion and injection in protection rates, which had the highest protection rate among the three immunization modes. On the contrary, regarding the impact of virus attack mode, oral attack had inferior protection rate compared to immersion, but was not significantly different from injection (Fig 4E).

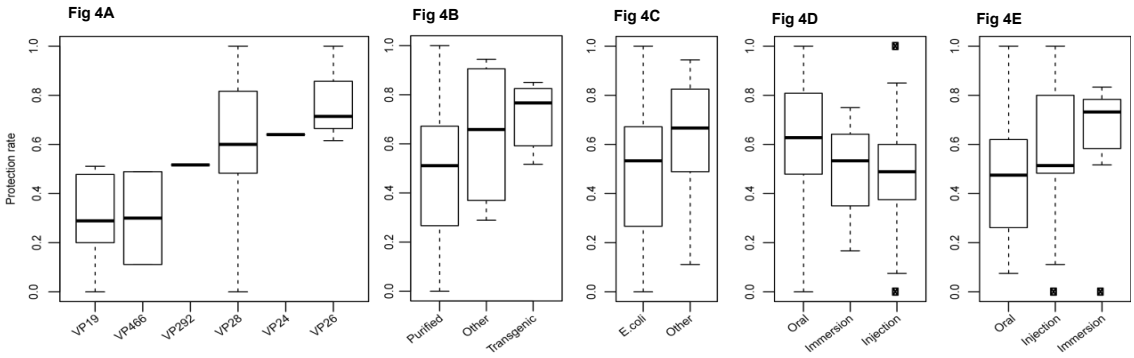

Fig. 4. Box plot of protection rates for studies grouped by different factors. (4A) Proteins; (4B) Protein forms; (4C) Expression hosts; (4D) Immunization mode; (4E) Virus attack mode.

3.3 The best practices for RNA-based vaccine

Compared to other types of vaccines, RNA-based vaccines had the best protection rate and were homogeneous across studies (Fig 1D and 1E). To find the best practices for RNA vaccines, a logistic regression mode was adopted to further investigate the impact of dsRNA target genes, immunization mode, and virus attack mode on protection rate of RNA-based vaccines. Compared to other genes, the

combination of PmRab7 and rr2 genes has the best protection rate of 95%. However, the differences in protective effects between PmRab7+rr2 and other genes investigated in those studies were not statistically significantly ($p=0.10$). Oral immunization performed significantly worse than injection ($p=0.03$) in immunization mode, while there was no significant difference observed between oral and injection virus attack mode ($p=0.3$).

4. Discussion

Through the first-time analysis with the meta tool, this study not only compared the protection rates of different types of vaccines, but also analyzed the protective effects of different protein subunit vaccines. Among the four types of vaccines, RNA-based vaccines have the highest protection rates over the other types of vaccines. The reason for this may be that RNA-based vaccines work directly at the transcriptional level, which targets the early stages of viral replication, not at the DNA or protein level [51, 52, 58]. Among the various subunit proteins vaccines, VP26 vaccine showed the best protective effect, not the most studied VP28 vaccine as might be expected. This may be due to the heterogeneity among studies of VP28 vaccine, as some studies had relatively lower protection rates and make the overall estimated average protection rate low. Multiple factors could have contributed to the variation of protection rate, e.g. immunization time, vaccine dose, and immunization route. However, the source of heterogeneity cannot be fully elucidated due to the limited details in the reports. This result needs to be further investigated by more comprehensive comparison that make the all other conditions consistent in the future vaccine studies.

Due to the fact of lack of effective routes of administration and immunization program, WSSV vaccine still has not been fully utilized in the actual production of aquaculture.For this reason, this study further investigates effects of some factors that potentially impact vaccine protection rate. Through comparison of eukaryotic system and prokaryotic system, the results demonstrated that protein vaccines expressed in eukaryotic hosts had a higher protection rate than that in prokaryotic *E. coli* [10, 25, 26]. Among the three immunization modes used in subunit vaccines, oral mode differs significantly from immersion and injection, and has the highest protection rate. This mode is also more suitable for operation and application of a vaccine in the practical breeding of shrimp. As to the virus attack mode, the results showed that the mortality caused by oral infection is similar to that of the injection route, but worse than that of the immersion mode of virus attack [7, 16, 17]. So, it implies that shrimps are primarily infected with the virus by oral means under natural conditions. Furthermore, it suggests that disinfecting the water environment is conducive to the prevention and control of WSSV.

In addition, the immunization dose of vaccine and the challenge dose of the virus have a crucial effect on the immune effect. However, since the specific immune dose or challenge dose is not well stated [14, 25, 55], or the unit of measurement is not consistent in many references [59, 60], these factors were not taken into account in the current study. With more comprehensive statistical data, the study can further investigate in more details. In general, establishment of a high-efficiency immune program could advance the progress of actual application of WSSV vaccines in shrimp farming, and provide reference for the control of other viral diseases in crustaceans.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgments

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